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α_1-Antitrypsin deficiency and anti-proteinase 3 antibodies in anti-neutrophil cytoplasmic antibody (ANCA)-associated systemic vasculitis

J. A. SAVIGE, L. CHANG, L. COOK*, J. BURDON*, M. DASKALAKIS† & J. DOERY† *University Department of Medicine, Austin Hospital, Heidelberg, *Respiratory Unit, St Vincent's Hospital, Fitzroy, and †Biochemistry Department, Monash Medical Centre, Clayton, Australia*

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SUMMARY

α_1-antitrypsin (α_1-AT) is a naturally occurring inhibitor of proteinase 3 (PR3) and elastase, two of the target antigens of anti-neutrophil cytoplasmic antibodies (ANCA). An increased incidence of α_1-AT phenotypes associated with dysfunctional α_1-AT or low serum levels has been reported in patients with anti-PR3 antibodies. We have studied the relationship between ANCA, and phenotypes and serum levels of α_1-AT. Phenotypes usually associated with a moderate or severe reduction in α_1-AT serum levels or in dysfunctional activity were found more often in individuals with anti-PR3 antibodies than in the general population: four of the 31 patients (13%) with anti-PR3 antibodies had phenotypes MZ ($n = 2$), S ($n = 1$) or Z ($n = 1$) ($P < 0{\cdot}05$). However, the corresponding α_1-AT serum levels were normal ($n = 3$) or elevated ($n = 1$). None of the 31 sera with anti-PR3 antibodies had low levels of α_1-AT. No abnormal α_1-AT phenotype was demonstrated in seven patients with anti-elastase antibodies, despite a low level of α_1-AT in one serum. Anti-myeloperoxidase antibodies are common in patients with ANCA, but no abnormal phenotype or low serum α_1-AT level was demonstrated in any of 29 sera containing these antibodies. Finally anti-glomerular basement membrane (GBM) antibodies occur occasionally in patients with ANCA-associated diseases, but again none of 10 sera had an abnormal α_1-AT phenotype or low serum level. ANCA were not demonstrated by indirect immunofluorescence in any serum from 73 patients with abnormal α_1-AT phenotypes. These results confirm that patients with anti-PR3 antibodies often have α_1-AT phenotypes that are usually associated with low serum levels of α_1-AT or with dysfunctional protein. Nevertheless, the incidence of anti-PR3 antibodies in patients with abnormal α_1-AT phenotypes is very low. This probably reflects the rarity of Wegener's granulomatosis, the major disease associated with anti-PR3 antibodies, and the relative frequency of abnormal α_1-AT phenotypes. The mechanism for the development of anti-PR3 antibodies in patients with abnormal α_1-AT phenotypes is not clear, but may relate to the increased propensity of unbound and uninhibited PR3 to stimulate autoantibody production.

Keywords α_1-antitrypsin ANCA elastase proteinase 3 vasculitis

INTRODUCTION

ANCA are antibodies directed against neutrophil cytoplasmic enzymes, including proteinase 3 (PR3), myeloperoxidase (MPO) and elastase. They are often associated with the presence of a systemic vasculitis [1]. Anti-PR3 antibodies occur in nearly all patients with active generalized Wegener's granulomatosis (WG) [2], while anti-MPO antibodies [3] and to a lesser extent anti-elastase antibodies [4], are found in patients with microscopic polyarteritis, other vasculitides and some unrelated diseases [5–7]. α_1-antitrypsin (α_1-AT) is the major naturally occurring inhibitor of PR3 [8.9] and one of the inhibitors of elastase, but it does not bind to MPO. There have been two reports that the α_1-AT phenotypes corresponding to dysfunctional inhibitor or low serum levels are more common in patients with anti-PR3 antibodies and the associated systemic vasculitides [10,11].

α_1-AT is encoded by a highly polymorphic gene with at least 75 alleles [12,13], that correspond to normal, moderate or severely deficient levels or abnormalities of function. While the most common association of dysfunctional or low levels of α_1-AT is with the development of emphysema [14,15] and liver disease, cutaneous and systemic vasculitides and a necrotizing glomerulonephritis [16–18] have also been recognized.

Correspondence: Dr Judy Savige, University Department of Medicine, Austin Hospital, Heidelberg, Victoria 3084, Australia.

The aims of this study were to confirm the association of anti-PR3 antibodies with abnormal α_1-AT phenotypes; to demonstrate any correlation between anti-PR3 antibodies and low levels of α_1-AT; and to determine how often anti-PR3 antibodies occur in individuals with abnormal α_1-AT phenotypes.

PATIENTS AND METHODS

Patients

Two groups were studied. Patients in Group A had glomerulonephritis, and antibodies against PR3 ($n = 31$), MPO ($n = 29$), elastase ($n = 11$), both MPO and elastase ($n = 2$), or glomerular basement membrane (GBM) ($n = 10$). α_1-AT phenotype and corresponding serum levels were determined for each patient.

Patients in Group B ($n = 73$) had been tested for α_1-AT phenotype because of suspicious clinical features, and were found to have a phenotype other than M. Phenotypes included those associated with a normal level of functioning α_1-AT (MS, $n = 14$), moderately deficient levels (MZ, $n = 33$, SZ, $n = 2$, S, $n = 1$), or severely deficient levels (Z, $n = 23$). Sera from these patients were screened for ANCA by indirect immunofluorescence (IIF), and any positive sera would be tested for anti-PR3 and anti-MPO antibodies in specific antigen ELISAs.

Anti-PR3 antibodies

High performance liquid chromatography (HPLC)-purified PR3 (Biocarb, Lund, Sweden) was coated to microtitre plates (Costar, Cambridge, MA) at 0·7 μg/ml for 18 h at 4°C. Sera were tested in triplicate at concentrations of 1/50 in PBS/Tween 20 (PBS–T; Sigma, St Louis, MO) and each assay contained negative and positive standards. Bound antibody was detected with alkaline-phosphatase-linked anti-heavy and light chain antiserum (KPL) diluted 1:500 in PBS–T, containing 0·1% bovine serum albumin (BSA; Sigma); the substrate used was *p*-nitrophenylphosphate (Sigma), 5 mg tablet in 5 ml 0·05 M carbonate buffer pH 9·6, with 0·02% $MgCl_2$. The amount of binding was determined in an ELISA plate reader at OD 405 nm (Titertek Multiscan, Finland). All incubations were for 1 h and plates were washed three times with PBS–T between incubations. Positive sera were defined as those that bound at levels greater that the mean + 4 s.d. of 33 normal laboratory workers.

Anti-MPO and anti-elastase antibodies

MPO (Calbiochem, La Jolla, CA) or elastase (Calbiochem) were coated to plates at a concentration of 0·5 μg/ml, and these assays were then performed as described for the anti-PR3 antibodies.

Anti-GBM antibodies

This assay has been described previously [19]. The non-collagenous region of type IV collagen that contains the Goodpasture antigen was obtained by collagenase digestion (collagenase type I; Sigma) of isolated GBM. This was coated to microtitre plates at a concentration of 10 μg/ml for 18 h at 4°C. Each serum was assayed in triplicate at a dilution of 1 : 8, but otherwise the assay was performed as described for anti-PR3 antibodies.

α_1-AT phenotypes

The α_1-AT phenotype was determined by isoelectric focusing [20]. Briefly, aliquots of frozen serum were thawed, 15 μl electrophoresed in an acrylamide gel using ampholytes in the range 4·2–4·9 (Pharmacia, Uppsala, Sweden). Electrophoresis was performed at a constant power of 20 W (300–1000 V) for 3 h. Protein bands were visualized after fixing and staining with coomassie blue R-250 overnight. After destaining the α_1-AT phenotypes were interpreted against known controls on the same gel.

α_1-AT levels

Levels of α_1-AT were assayed in thawed frozen sera using 20 μl of serum with 40 μl of antibody against α_1-AT (Behring, Marburg, Germany) in a Behring Nephelometer system using a fixed time rate measured between 10 s and 360 s. The assay was calibrated using dilutions of Human Serum Calibrator 1 (Incstar Corporation, Stillwater, MN). The normal range was calculated from the mean ± 2 s.d. of levels from 50 healthy male and 50 healthy female blood donors.

ANCA

ANCA were demonstrated by IIF examination of normal peripheral blood neutrophils using the standard method of the First International Workshop on ANCA [21], and each assay included a negative and positive standard.

Statistical analysis

The associations between the presence of a deficient phenotype and anti-PR3 or other antibodies were tested with χ^2 analysis.

The distribution of α_1-AT phenotypes in the Australian population is approximately: normal phenotypes PiMM 88·6% and PiMS 8·0%; moderately deficient phenotypes PiMZ 1·5%, PiS 0·3% and PiSZ 0·2%; and severely deficient phenotypes PiZ 0·03%.

RESULTS

Four patients of the 31 with anti-PR3 antibodies (13%) had a phenotype associated with a moderate deficiency (3/31, 10%) or severe deficiency (1/31, 3%) of α_1-AT (Table 1). This represents a significant increase in incidence of α_1-AT phenotypes associated with moderate and severe inhibitor deficiencies in patients with anti-PR3 antibodies and a systemic vasculitis compared with the normal population ($\chi^2 = 4{\cdot}46$, $P < 0{\cdot}05$). The remaining 27 patients with anti-PR3 antibodies (87%) had α_1-AT phenotypes not associated with deficient α_1-AT levels nor abnormalities of function (M, MS or DM).

No serum from any of the 26 patients with anti-PR3 antibodies and a systemic vasculitis had a reduced level of α_1-AT. Levels were normal in 12 individuals (46%), or elevated in 14 (54%) (Table 2). These included sera from patients with MZ ($n = 2$), S ($n = 1$) or Z ($n = 1$) phenotypes, whose levels of α_1-AT were within the normal range ($n = 3$) or elevated ($n = 1$).

No α_1-AT phenotype associated with dysfunctional or reduced levels of α_1-AT was demonstrated in any serum with anti-elastase, anti-MPO, both anti-elastase and anti-MPO, or anti-GBM antibodies. Levels of α_1-AT were normal or elevated in all of these sera, except one with anti-elastase antibodies associated with a normal α_1-AT phenotype. Elevated levels of

Table 1. α_1-Antitrypsin phenotypes in patients with ANCA, anti-glomerular basement membrane (GBM) antibodies and the normal population

	α_1 anti-trypsin phenotype					
	Non-deficient			Moderately deficient		Severely deficient
	M	MS	DM	MZ	S	Z
Normal population	87%	8%	0·7%	1·5%	0·3%	0·03%
Antibodies						
Anti-proteinase 3 ($n = 31$)*	23 (74%)	3 (10%)	1 (3%)	2 (6%)	1 (3%)	1 (3%)
Anti-myeloperoxidase ($n = 29$)	27 (93%)	2 (7%)	0	0	0	0
Anti-elastase ($n = 11$)	11 (100%)	0	0	0	0	0
Anti-myeloperoxidase plus anti-elastase ($n = 2$)	2 (100%)	0	0	0	0	0
Anti-GBM ($n = 10$)	10 (100%)	0	0	0	0	0

* Association between anti-proteinase 3 antibodies and abnormal α_1-antitrypsin phenotypes $P < 0.05$. No other significant associations.

α_1-AT were more common in patients with anti-GBM disease (7/8) than in any other antibody group. ANCA were not demonstrated by IIF screening in any serum from 73 patients with α_1-AT phenotypes, usually associated with dysfunctional or deficient α_1-AT.

DISCUSSION

We have confirmed an increase in abnormal α_1-AT phenotypes in patients with anti-PR3 antibodies compared with the normal population. However, the incidence and severity of these phenotypes is less in our study than described previously [10,11], where seven of 14 patients (50%) with anti-PR3 antibodies had the severely deficient Z ($n = 3$) or moderately deficient varieties MZ, IS and MS ($n = 4$). The distribution of abnormal α_1-AT phenotypes in any population depends on its racial origin, but there is no apparent difference between the incidences in this European population and the general Australian population.

In one study where there was an association between abnormal α_1-AT phenotypes and anti-PR3 antibodies, levels of serum α_1-AT were significantly lower in patients with systemic vasculitis and pulmonary haemorrhage, than in those without haemorrhage or in normal individuals [22]. Thus α_1-AT deficiency appeared to modify the expression of the systemic vasculitis. In another study, abnormal α_1-AT phenotypes were associated with more severe disease [23]. We have not determined the clinical features of the patients in our report.

We have not shown low levels of circulating α_1-AT in patients with anti-PR3 antibodies, even in those individuals with abnormal phenotypes. All patients with anti-PR3 antibodies including those with abnormal α_1-AT phenotypes had normal or elevated serum levels of α_1-AT. This elevation reflects the degree of inflammation and the role of α_1-AT as an acute phase reactant, and it has been suggested that elevated levels of α_1-AT may indicate disease activity as effectively as levels of anti-PR3 antibodies or C reactive protein [24].

α_1-AT is also an inhibitor of elastase, but there was no increase in abnormal α_1-AT phenotypes in patients with anti-elastase antibodies in our study. In one patient with anti-elastase antibodies and a normal phenotype, the level of serum α_1-AT was low, but the α_1-AT may have been denatured by repeated freezing and thawing.

Anti-MPO antibodies are often associated with microscopic polyarteritis, which shares many clinical and laboratory features with WG. However, there was no increase in abnormal α_1-AT phenotypes, and levels of the α_1-AT in patients with anti-MPO antibodies were normal or elevated in our series. There has been a report of an association of abnormal α_1-AT phenotypes in patients with anti-MPO antibodies and microscopic polyarteritis: six of the 11 patients had MZ or MS phenotypes usually associated with moderate α_1-AT deficiencies [10]. However, this report was from the same group who

Table 2. α_1-Antitrypsin levels in patients with ANCA, and anti-glomerular basement membrane (GBM) antibodies

	α_1-antitrypsin level		
Antibodies	Low (%)	Normal (%)	High (%)
Anti-proteinase 3 ($n = 26$)	0 (0)	12 (46)	14 (54)
Anti-myeloperoxidase ($n = 29$)	0 (0)	22 (76)	7 (24)
Anti-elastase (= 7)	1 (14)	5 (71)	1 (14)
Anti-myeloperoxidase plus anti-elastase ($n = 2$)	0 (0)	11 (50)	1 (50)
Anti-GBM ($n = 8$)	0 (0)	1 (13)	7 (87)

Normal range was 1·5–2·6 g/*l*.

have shown a much higher incidence of abnormal phenotypes in patients with anti-PR3 antibodies than we have seen.

We have not been able to demonstrate ANCA in any of the individuals with abnormal α_1-AT phenotypes demonstrated in response to a clinical request and usually associated with moderately ($n = 36$) or severely deficient ($n = 23$) levels of inhibitor. In contrast, in one other study, 20 out of 150 individuals (13%) with the severely deficient phenotype Z had ANCA [10]. These included 12 C-ANCA and eight P-ANCA and surprisingly the antibodies were directed not only against PR3 and elastase, but also against other enzymes that are not inhibited by α_1-AT.

Any explanation as to why anti-PR3 antibodies occur in patients with α_1-AT phenotypes must take into account the observations that anti-PR3 antibodies are found in patients with abnormal phenotypes but with normal α_1-AT levels, that in most patients the levels of α_1-AT are increased, and that antibodies directed against other neutrophil enzymes not inhibited by α_1-AT possibly also occur more frequently in these patients.

Why anti-PR3 antibodies occur more often in patients with abnormal α_1-AT phenotypes is not clear. α_1-AT is usually complexed to PR3 in the circulation and in secretions [25]. It binds to the catalytic site of PR3 and can inhibit binding of this enzyme to its substrate and thus its enzymatic activity [22]. Individuals with deficient or abnormal α_1-AT may have a PR3 molecule whose catalytic site is exposed and against which autoantibodies are produced in the appropriate immunologic environment. We have not found an increase in abnormal phenotypes in patients with anti-elastase antibodies. The explanation for this apparent lack of association may be that there are other more significant inhibitors of elastase than α_1-AT, or that α_1-AT has a lower affinity for elastase than for PR3, or that α_1-AT does not not bind to the catalytic site of elastase.

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